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Survival Studies of Bacterial Pathogens And Their Immunization Effect on Fishes (*Channa marulias and Clarias batrachus*) in Glass Aquaria.

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ABSTRACT
Present study was carried out to examine for the growth and survival of *Channa marulias and Clarias batrachus* cultivated in glass aquaria. An experiment was conducted in four glass aquaria (size 90 × 30 cm) for a period of 21 days in October 2018. 10 fishes of same size (age group) of *Channa marulias and Clarias batrachus*, with mean initial length and weight of 6.5 ± 0.07cm and 5.8 ±0.04 g respectively were assigned to each aquaria. The aim of this work is to determine the concentration of bacterial pathogens to be inoculated in *Channa marulias and Clarias batrachus*, so as to induce bacterial infection but not death during a phase of at least two days and, therefore, allow the development of treatment protocols. The clinical exam was done 24 h after inoculation, and the clinical signs suggested bacterial infection in all fishes. In the lowest concentration, fishes demonstrated few clinical signs of disease, and in the highest concentration (4.5 x 106 CFU/ml), all fishes died within 24 - 48 hrs of bacterial induction with acute infection.

Keywords: *Channa marulias, Clarias batrachus,* Bacterial pathogens.

INTRODUCTION

We use catfish *Clarias batrachus* [1]. It is highly nourishing and valued as food. It is mostly used in laboratories for experimental purposes but also used as a food. The soft tissue has high nutritive value and its flesh is said to have wound healing result and recuperative attributes.

It is extremely suitable for intensive culture due to its air-breathing routine. In central India it is commonly found in reservoirs of eastern Vidarbha region (M.S.). It is an earlier growing fish than mainly of the other species of the genus. It is marketed be alive and obtain high prices in the market.

Channa morulias is native to South Asia. In South India it is commonly found in reservoirs of eastern Vidarbha region. It is a quicker growing fish than most of the other species of the genus. It is a carnivorous species. It is marketed be alive and fetches high prices in the market. The fish *Channa morulias* is well known for its nutritional value. A well identified economic loss to the fish industry was the major outbreak of bacterial infection in major carps. Healthy fishes are prized for their table quality.

However, this significance is influenced by several operational environmental factors. Suggested to the type of micro-organisms that are initiate associated with particular fish depends on its habitat classified the bacterial pathogens associated with fish as indigenous and non-indigenous. The non-indigenous contaminate the fish or the habitat one way or the new and examples include *Escherichia coli, Clostridium botulinum, Shigella dynteriae, Staphylococcus aureus, V. cholera* and *Salmonella*. The infectious agents of the severe acute infectious of abdominal dropsy outbreak in Indian major carps. *Cirrhinus mrigala* was reported Shome et al[2]. However, the first examination on diseases in Indian major carps was establish in descending order of susceptibility on *Catla catla*, *Cirrhinus mrigala* and *Labeo rohita*[3]. Further well recorded cases have been the severe epidemic due to the diseased form of European carps.

Microbial fish diseases causes considerable economic losses in aquaculture yearly and it represents a worldwide problem. Members of the genus *Vibrio* are the causative agents of vibriosis, which can cause significant losses in fish culture. *Vibrio* spp. causes disease in many fish as well as the salmon, char and shellfish such as the shrimp. *Escherichia coli* is a common human pathogen, contaminants of seafood in enormous number and fish usually acquire this pathogen through feeding on food contaminated with feces causing serious life threatening disease within a very short moment.

In the present work, experimental infection was done to know the pathogenicity of bacterial pathoens in *Clarias batrachus and Channa morulias*. The virulence of the pathogen was predictable by experimental studies of the LD_{50} (median lethal dose) of *bacterial pathogens* in the glass aquaria.

METHODOLOGY

Study Area

This work was conducted on fishes species collected from Wainganga River flowing through Gadchiroli and Chandrapur district, Vidharbha (M.S.) In Gadchiroli district the river flows nearby Armori tehsil and in Chandrapur district it is near Bramhapuri tehsil.

Sampling

The bacterial counts on the exterior surfaces, intestines and tissue were predictable as follows:

Skin Surfaces

Sample of fish species from different locations of the skin of 30 raw fish was taken by rubbing the sterilized cotton swab over the skin and then inoculated into 9ml of Nutrient broth, MacConkey broth which are dispensed in separate tubes. Serial dilution of the bacterial suspension inoculated in peptone water was arranged in duplicate and viable aerobic bacterial counts were enumerated using 0.1ml and 1ml inoculums in standard plate count agar as described by (Slaby, B.M., Martin, R.E., Ramsdell, G.E.1981)[4], and then incubated at 37°C for 48 hrs.

Intestines, Gills and Tissues

1 gm of the each fish sample was dissected out, blended and mixed properly in a mortar. It was sample transferred aseptically to bottle containing 9 ml of 0.1% sterile peptone water. The bottle was closed and shaken thoroughly for 10 minutes and allowed to stand for 20 – 30 minutes, after which a 10 fold serial dilution was carried out in duplicates and viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37°C for 48 hrs as described by [4]. Coliforms organisms and

Gram negative enteric bacteria counts were determined by pour plate method with MacConkey agar, EMB Agar respectively. Mueller-Hinton Agar for *Pseudomonas spp*. *Salmonella spp*. and *Shigella spp*. is enumerated using Salmonella Shigella Agar (SSA) and Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar for pathogenic *Vibrio spp*. The plates were incubated at 37°C for 24hrs. The experimental colony growth were counted using Coulter™ Colony counter according to plate count method. Identification of the organisms was done using the biochemical characteristics as described by [5].

Estimation of mean colony forming unit per gram (CFU g1)

The mean of colony forming unit per gram (CFU g-1) denoted by (x) was calculated as $\Sigma f\chi/\Sigma f$, where Σfx is the sum of the products of number of colonies and the colony forming unit per gram; while Σf is the summation of the number of colonies.

Collection and Stocking of Fingerlings

The experimental fingerlings of *Channa marulius* and *Clarias batrachus* were collected from Wainganga River flowing through Gadchiroli and Chandrapur district, Vidharbha (M.S). For the feed check test 04 glass aquaria (size 90 × 30 cm) were selected for a period of six months starting from January to Jun 2014. Two feed administrations (treatments) i.e. Feed A with 40%, Feed B with 39.40% (gross protein) were replicated twice. The sample fish belongs to the same age group having mean length and weight of 6.5 ± 2.20 cm and were stocked at the rate of five fish/aquaria.

Median lethal dose (LD_{50}) experiment

An amount of 10 mg of fresh fish culture of the bacteria was carefully scraped and mixed with1 ml sterile physiological saline (PS) and desired dilutions were prepared by serial decimal dilution method. In a beginning test the above stock dilution (10 mg in 1 ml) was calculated to contain around 10^6 CFU/ml. Serial dilutions having an estimated concentration of 10^7, 10^6, 10^5 and 10^4 CFU/ml were used for the (LD_{50}) experiment. Since each of the above 4 dilutions, 0.1 ml bacterial suspension was injected intramuscularly to each of previously stocked and acclimatized 10 fish making a group. Every group was then released in one aquarium properly labeled to know the dose. The injected fish sample was observed up to 21 days. No nourish was given to the experimental fish and water temperature was recorded twice daily. without delay after death, each fish was transferred to laboratory; kidney was dissected out, touched with a sterilized loop and streaked onto TSA plates. The plates were incubated at 25°C for 48 hours for colony appearance. From the mortality record, LD_{50} value was worked out according to the following formula:

Proportionate distance (PD) =

50% mortality - mortality at dilution next below 50%

Mortality at dilution next above 50% - mortality at dilution next below 50%

RESULTS AND DISCUSSION

Median lethal dose (LD_{50}) for *Channa marulius*

The pathogenisity tests are shown in Table No. 1. of *Channa marulius* was proved to be sensitive to bacterial species as shown by their mortality upto 100%, at a dose of 4.5 × 10^6 CFU/ml shown by *Pseudomonas aeruginosa* and *Salmonella typhi* compared to 4.5× 10^5 CFU/ml contain 60% and 80% respective bacteria. While, maximum mortality of *Staphylococcus aureus, Escherichia coli., Streptococcus pneumoniae, Vibrio cholerae* and *Shigella dysenteriae* shown that 80%, 60%, 80% and 80% respectively in 4.5 × 10^6 CFU/ml dilution factor compared to 4.5× 10^5 CFU/ml contained 40%, 40%, 40% and 40% respected pathogens during 2-6 days of period.

Median lethal dose (LD_{50})

Results of LD_{50} test are presented in Table 2. All the fish died with 4.5 × 10^6 CFU bacteria/fish within 2 - 5 days. With the dose of 4.5 × 10^5 CFU/fish, 3-4 fish died out of 5. Among them three fish died at the day of doses, one fish died at 2nd day, one fish died at 4th day of doses. In the case of 4.5 × 10^4 CFU/fish, 2 fish died out of 5. Among them one fish died at 3rd day and another fish died at 6th day. In this case, streaking and incubation from each dead fish gave rise to the appearance of pure colonies of bacterial pathogens.

Results of LD_{50} test are presented in Table No. 1 and 2 shown that all the fish died with 4.5 × 10^6 CFU /ml

within 2 - 5 days, among them 2 fish died at the day of doses, 1 fish died at 2nd day, 1 fish died at 5th day and 1 fishes died at 6th day. With the dose of 4.5×10^5 CFU/ml, 4 fishes died out of 5. Among them 1 fish died at the day of doses, 1 fish died at 2nd day, 1 fish died at 4th day and 1 fishes died at 6th day. In this case, streaking and incubation from each dead fish gave rise to the appearance of pure colonies of bacterial pathogens.

The study proved that bacterial pathogens, though opportunistic, was a serious pathogen for human beings. It was found that the pathogenesis of the pathogen was very active at least in liver, kidney and intestine of the experimental fish, investigated. As a everywhere species, of bacteria are available in water, fish body, and other aquatic animals and even in their feed. From the above discussion it is clear that the pathogen might be an important disease causing agent of fishes in Chandrapur and Gadchiroli (M.S.) aquaculture. Generally pathogenic bacteria are found to cause disease in fishes associated with fungus to produce EUS[6].

As a bacterial pathogen, it is causing severe losses of fish by falling fish production and ultimately hampering the national economy. It has been isolated from lesions of almost all infectious diseases. So, proper preventive as well as curative measures should be taken for the reduction of the disease conditions caused.

DISCUSSION

An elevated population of bacteria in food indicates the general quality of the food and the degree of spoilage it might have undergone. The amount of total bacterial counts of many of the samples investigated having > 5 X 10^6 CFU/g raises concern about the hygienic status of the production and point of sale environment. The results from this study and according to published microbiological guidelines as cited by suggest that the microbiological quality of the fish examined is unacceptable and pose a potential risk to public health. The range of potential pathogens from the samples of fish is of concern particularly at a time when many in our communities are immunologically compromised as a result of various illnesses. These opportunistic and pathogenic bacteria were also previously isolated by several other researchers from fish [7].

The fish studied harbored human disease causing organisms that cause diseases such as food poisoning, diarrhea, typhoid fever and Shigellosis [8]. Suggested that when, pathogens such as *S. aureus, Salmonella, Shigella* and *Pseudomonas* are the majority likely to cause food-borne diseases. The elevated incidence of *Salmonella* in the fish from the river is a major health concern.

The isolation of *Salmonella, Shigella* and *E. coli* point out faecal and environmental pollution. Coliforms such as *E. coli* are usually present where there has been faecal contamination from warm blooded animals. The organism *E. coli* is recognized as the reliable indicator of faecal contamination in small numbers and in large number sit is an indicator of mishandling. In comparable studies, *Escherichia coli, Pseudomonas aueriginosa, Shigella dysenteriae, Staphylococcus aureus* and *Salmonella typhi were* isolated from the gills, intestines. This was recognized to the heavy load of sewage disposal into the seas which could act as a suitable environment for the growth and survival of the human pathogens.

CONCLUSION

Seven human bacterial pathogens i.e. *Escherichia coli, Pseudomonas aueriginosa, Shigella dysenteriae, Staphylococcus aureus, Vibrio cholerae,* and *Salmonella typhi* were isolated from the two fish species *Channa marulias* and *Clarias batrachus* collected from Wainganga river of Gadchiroli District. The presence, in large populations of these bacterial pathogens indicates high levels of faecal contamination in the river. The presence of enteric bacteria may be attributed to faecal contamination due to improper sewage disposal and or water pollution. The fish act as a reservoir of human pathogens and the presence of highly pathogenic agents such as *Salmonella, Shigella* species and of opportunistic pathogens is a potential health risk/hazard to human beings and may cause diseases to susceptible individuals especially the immune-compromised consumers. Moreover the recoveries of various organisms, which are potentially pathogenic to humans, in the fish suggest that if they

are improperly handled, undercooked or consumed raw may contribute to the spread of the pathogens in the community. Further examination of fish especially for the presence of pathogens, during handling, storage and up to the very point of consumption is needed for the protection and maintenance of community health by keeping food borne diseases to a minimum.

As the bacteria are species specific parasites, it was found from the present study that bacteria are highly pathogenic to fresh water ornamental fish *C. marulias* and *Clarias batrachus* causing parasitism. There may be certain toxins present in given species of bacteria which cause pathogenesis in fish lead to change in hematological parameters and varying degree of destruction in the tissue which leads to mortality of fish.

Conflicts of interest: The authors stated that no conflicts of interest.

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